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JCAD – From systems genetics identification to the experimental validation of a coronary artery disease risk locus

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GWAS to identify coronary artery disease risk variants

Coronary artery disease (CAD) is one of the leading causes of mortality in the world and triggered by different risk factors, including diet, dyslipidaemia, diabetes, inflammation, and – importantly – also genetics[1, 2]. Indeed, heritable factors account for 40% to 60% of the risk to develop CAD[1], and genome wide association studies (GWAS) have the potential to identify genetic variations associated with CAD.

The first GWAS for CAD were published in 2007[3], which initiated an exciting era of cardiovascular systems genetics (also described as systems medicine) to detect new loci associated with cardiovascular complications[4]. Although several follow up studies led to the identification of various genetic polymorphisms that are associated with increased incidence of CAD or myocardial infarction (MI), including single nucleotide polymorphisms (SNPs), these identified variants only explain approximately 10% of the risk heritability[4].

One of the best ways to validate the cardiovascular function of genetic variants is to study their role in genetically modified model organisms[5, 6]. Importantly, a systematic review from von Scheidt et al. demonstrated that most CAD risk variants from GWAS display consistent phenotypes in experimental atherosclerosis mouse models[7].

JCAD – a new atherogenic player?

Several GWAS studies have identified genetic variants at the *junctional cadherin 5 associated* (*JCAD*, also known as *KIAA1462*) locus to be associated with increased risk of CAD and MI, including rs3739998, rs2505083, and rs2487928[8-11]. Recently, rs2487928 has been shown to reduce *JCAD* expression, which via the Hippo pathway has downstream consequences on a number of downstream phenotypes including angiogenesis[12]. In this issue of the *European Heart Journal*, Xu et al. (new REF) mechanistically examine this interesting question by characterizing *Jcad* knockout mouse models, performing vascular function studies, and assessing how *JCAD* modulation affects atherosclerosis development (**Figure 1**).

Risk variants at the *JCAD* locus lead to increased *JCAD* expression

The authors initially determined the magnitude and directionality of the association between *JCAD* risk variants and CAD by mining genotype data from GWAS and expression data from the Genotype-Tissue Expression (GTEx) database. They observed that the lead risk variants associate with increased *JCAD* expression levels (**Figure 1A**), that JCAD is highly expressed in endothelial-enriched organs, such as arteries, lungs and brain, and that the disease-associated *JCAD* variants map to eQTLs most strongly in arterial tissues—particularly aorta. Given this association between CAD-risk variants and increased *JCAD* expression, the authors hypothesized that the deletion of *Jcad* would exert atheroprotective effects in mice.

Systemic deletion of *Jcad* improves vascular function in mice

To test their hypothesis the authors first characterised whole-body *Jcad* knockout mice. When challenging the mice with a high-fat diet, *Jcad* knockouts displayed an improved endothelial-dependent vascular relaxation compared to wildtype controls. Surprisingly, although JCAD is a structural membrane protein, vascular permeability was not affected in *Jcad* knockout mice.

Systemic and endothelial cell-specific deletions of *Jcad* protect against atherogenesis in mice

To further validate the contribution of JCAD to atherosclerosis, the authors crossbred whole-body and endothelial cell-specific *Jcad* knockouts with atherosclerosis-susceptible *Apoe*$^{-/-}$ mice. Both mouse models with *Jcad* deficiency developed less atherosclerotic lesions compared to the control mice upon high-fat diet feeding. While there were no notable differences in blood lipid levels, smooth muscle cells, collagen content or inflammation, the authors demonstrate that macrophage staining is reduced in plaques. Consistently, VCAM-1 expression and monocyte adhesion to mouse endothelial lung cells were reduced in *Jcad* knockout mice (**Figure 1B**).

An atheroprotective JCAD-YAP-TAZ axis in endothelial cells

Complementary RNA-sequencing studies in coronary artery endothelial cells showed that the expression of various pro-atherogenic genes was reduced, e.g. *CTGF* and *CYR61*[13, 14], while the expression of atheroprotective genes was increased upon *JCAD* siRNA-mediated silencing. CTGF and CYR61 are transcriptional targets of the Hippo/YAP/TAZ pathway, further reinforcing this as the mechanistic pathway connecting JCAD to atherosclerosis. The authors performed further gain and loss-of-function experiments in endothelial cells (HUVECs) to demonstrate that JCAD interacts with several actin-binding proteins and activates the Hippo/YAP/TAZ pathway (**Figure 1C**), thus leading to the nuclear translocation and transcriptional activation of the complex, and consequently *CTGF* and *CYR61* expression.

Regulation and expression of JCAD in human endothelial cells and atherosclerotic plaques

How is JCAD itself regulated? The authors tested different atherogenic, angiogenic and biochemical stimuli, and showed that the expression of *JCAD* is regulated by the blood flow, characterized by increased expression in areas with disturbed flow. Finally, the authors reveal that JCAD expression is increased in the aortic endothelium of mice upon high-fat diet feeding as well as in advanced human atherosclerotic plaques.

Added value and open questions

The current study of Xu et al. demonstrates that the deletion of *Jcad* is atheroprotective in mice and suggests that JCAD expression is increased in advanced human plaques. Together with the initial GWAS findings which indicate a causal relationship between *JCAD* and the incidence

of CAD in humans, this study highlights a potential new avenue for understanding and treating arterial diseases. Nevertheless, some questions remain open for future investigations, such as: Is the deletion of endothelial cell *Jcad* sufficient to improve vascular function in mice? This question could be easily addressed with the endothelial cell-specific knockout mouse model. How does blood flow regulate *JCAD* expression? As described by Jones et al., the RhoA signalling pathway might play a central role in this shear stress response[12]. Do the genetic variants that were identified in human GWAS lead to the same phenotype as the *Jcad* knockout mice? This last question could be addressed by using the CRISPR/Cas9 system to introduce the corresponding SNPs in an atherosclerotic mouse model and then assess atherosclerosis development.

In a compound screening approach, the authors identified the BRD4 inhibitor JQ1 as a pharmacological inhibitor of *JCAD* expression. Notably, JQ1 protects against inflammation and atherosclerosis development[15]. It is therefore tempting to speculate that JQ1 may also confer atheroprotection by repressing the expression of *JCAD*, which would be of high pharmacological interest.

Mechanistic understanding of GWAS hits

GWAS have been established as a powerful tool for the *de novo* identification of genetic mechanisms affecting complex diseases, yet this approach has been beset by challenges related to the mechanistic understanding of resulting hits. The authors show here a strong example for validating GWAS targets by using a pipeline going from hypothesis discovery in human populations, to mechanistic examination in cell lines, then to genetically modified mice. As *JCAD* is shown to have a strong impact on atherosclerosis and likely other arterial-related diseases, the authors have established a clear path forward both for additional mechanistic understanding, and for clinical examinations in humans.

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Conflict of interest

None declared

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Figure legend

Figure 1. Scheme illustrating the CAD risk variants at the *JCAD* locus and the role of JCAD in experimental atherosclerosis. **(A)** Using a systems genetics approach Xu et al. demonstrate that the CAD risk variants at the *JCAD* locus lead to increased *JCAD* expression. **(B)** JCAD promotes the activation of the YAP/TAZ pathway and subsequent expression of pro-inflammatory genes. Consequently, endothelial cells express higher levels of VCAM-1 and attract blood monocytes. **(C)** *Jcad*-deficient mice develop less atherosclerotic lesions that are characterized by a lower accumulation of macrophages and have an improved endothelial-dependent vascular function. CAD, coronary artery disease; EC, endothelial cell; GTEx, Genotype-Tissue Expression database; GWAS, genome wide association study; HFD, high-fat diet; Mono, monocyte; Recomb. rate, recombination rate.